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Bortezomib Aqueous Solubility in the Presence and Absence of D-Mannitol: A Clarification with Formulation Implications

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ABSTRACT

The solubility of bortezomib, a boronic acid, in water and normal saline is often misquoted in the literature. Here we confirm that bortezomib equilibrium solubility in water and normal saline is 0.59 ± 0.07 and 0.52 ± 0.11 mg/mL, respectively. The aqueous solubility is significantly enhanced, 1.92 ± 0.14 and 3.40 ± 0.21 mg/mL, respectively, in the presence of 55 mM and 137 mM D-mannitol in normal saline, as used in the commercial formulation of Velcade®. This is due to reversible ester formation. Based on the pH-solubility profile curve for bortezomib, its pKa value is estimated to be 8.8 ± 0.2. Boric acid, glycine and a combination of the two, used in an alternative formulation to that of Velcade® do not enhance the equilibrium aqueous solubility of bortezomib.

INTRODUCTION

The purpose of this short paper is to clarify some misinformation in the literature on the aqueous solubility of the bortezomib[1,2] as reported in various sources.[3-5] Much of the confusion comes from information listed in the package insert for Velcade®, the commercial freeze-dried formulation of bortezomib from Millennium/Takeda, which states that "the solubility of bortezomib as the monomeric boronic acid, in water is 3.3 to 3.8 mg/ml in a pH range of 2 to 6.5".[3] It also states that "the drug substance exists in its cyclic anhydride form as a trimeric boroxine".

In various patents describing the Velcade® formulation and the role that D-mannitol plays in the development of the safe, chemically stable and readily reconstituted product, Gupta et al.,[6,7] quoted a solubility for bortezomib from the freeze-dried formulation (from D-mannitol) of up to 6 mg/mL after reconstitution with normal saline (NS), whereas bortezomib itself "was *not* soluble in 0.9% w/v saline at a concentration of 1 mg/mL."

Velcade® can be reconstituted for intravenous (IV) administration to 1 mg/mL and 2.5 mg/mL for subcutaneous (SC) administration with NS, respectively.[3] Recently, FDA approved a bortezomib freeze-dried formulation alternative to Velcade®, that utilizes boric acid and glycine in place of D-mannitol and can also be reconstituted to 1 mg/mL allowing it to be given IV but cannot be reconstituted to the 2.5 mg/mL needed for SC administration (Fresenius Kabi LLC).[8]

The structures of bortezomib, its boroxine, D-mannitol and the structure of the D-mannitol ester of bortezomib are illustrated in Fig. 1.

Insert Figure 1

Reported here are various studies describing the equilibrium (and kinetic) solubility of bortezomib from two commercial batches in purified water (water) and NS (0.9% NaCl), and at varying pH values as well as the equilibrium solubility of bortezomib in the presence of up to 500

mM D-mannitol. Also presented is the solubility in the presence of boric acid, glycine, and boric acid plus glycine at the same concentrations used in the recently approved alternative formulation to Velcade®.[8]

EXPERIMENTAL

Materials

Boric acid (reag. Ph. Eur., ≥99.8%) and glycine (PharmaGrade), sodium chloride (Ph. Eur.), hydrochoric acid, (HCl) 37% ACS reagent, sodium hydroxide (NaOH) pure pellets, potassium dihydrogen phosphate, KH_2PO_4 (ACS reagent) and H_3PO_4 (ACS reagent, ≥85 wt. % in water) were commercially sourced from Merck (Milan, Italy). Acetonitrile (ACN), methanol (HPLC grade) and purified water (Ph. Eur.), water, were purchased from Carlo Erba s.r.l. (Milan, Italy). D-mannitol (Ph. Eur. powder) was purchased from Farmalabor s.r.l. (Canosa, Italy). Bortezomib (99%), was purchased from ABBLIS chemicals LLC (Houston, TX, USA).

Methods

High-Performance Liquid Chromatography (HPLC)

An Agilent1260 Infinity Quaternary LC System equipped with an Agilent variable wavelength UV detector, a Rheodyne injector (Rheodyne, Model 7725i, Agilent) equipped with a 20 µL loop and an OpenLAB CDS ChemStation software (Agilent) were used for HPLC analysis. A YMC octyl packed column (120Å, 5 µm, 4.6 x 150 mm) thermostated at 20°C was used. An isocratic separation was performed using 30 mM KH_2PO_4/H_3PO_4 (pH 3.0) and ACN (60/40 v/v) as mobile phase at a flow rate of 1 mL/min. An injection volume of 20 µL was used in all experiments.

Bortezomib was detected at 280 nm and quantitated from a linear relationship between the peak area and concentration. The total acquisition time was 10 min.[9]

pH-Solubility Determination

An excess of bortezomib (10 mg) was added to 2 mL of acidic or basic solutions and titrated to the desired pH using 200 mM HCl and NaOH without and with 500 mM of added D-mannitol. Each sample was left in an orbital shaking incubator at 25ºC and 50 rpm, over 24 hours. The second day aliquots of 1 mL were taken, placed in 2 mL polypropylene tubes, and centrifuged at 13,200 rpm for 10 min at 25ºC, using a Hettich Mikro 22R cooling centrifuge with fixed-angle rotor 1153 (LabMakelaar Benelux BV, Zevenhuizen, The Netherlands). The supernatant, 400 μL, of each sample was taken and placed in a 2 mL polypropylene tube. The pH of these solutions was monitored with a Mettler Toledo pH meter model SevenExcellence™ combined with a Mettler-Toledo InLab® Expert Pro-ISM electrode (Milan, Italy). Each aliquot was then diluted with purified water. Each sample was then analyzed by HPLC as described above.

Solubility in the presence of D-Mannitol

Phase solubility analysis was performed in water and 0.9% NaCl NS with an excess of bortezomib (10 mg/mL). To these samples, weighed solid D-mannitol was added to give final D-mannitol concentration in a range between 0 and 500 mM. Each sample was equilibrated in an orbital shaking incubator at 25ºC and 50 rpm, over 24 hours and then the supernatant analyzed by HPLC as described above.

Differential Scanning Calorimetry (DSC)and Thermogravimetric Analysis (TGA)

DSC and TGA experiments of bortezomib were performed as described previously.[8] Briefly, all DSC experiments were performed on a Perkin Elmer Pyris 1 Instrument DSC (US instrument division, Norwalk, CT). DSC samples were placed in an aluminum pan, sealed (unvented), and

the temperature ramped at a rate of 20ºC/min from ambient temperature to 300ºC. TGA experiments were performed on a Perkin Elmer Pyris 1 TGA 7 (US instrument division). Samples were placed on a clean platinum pan, supported by a platinum stirrup, at a weight of 3-5 mg per sample and ramped, in a dry nitrogen atmosphere, at a rate of 20°C/min to a final temperature of 300ºC.

Data Analysis

Data fitting to determine the solubility and Ka values, and thus pKa, of bortezomib and its presumed monoester was performed using GraphPad/Prism version 5.0 (GraphPad Software, La Jolla, CA) using nonlinear least-squares regression analysis. For the solubility fits, a 1/Y weighting was used. Standard deviation values are reported where possible.

RESULTS AND DISCUSSION

The solubility of bortezomib was first determined in our laboratory after it was first assigned as an active project through a formulation contract with the National Cancer Institute in 1997. In reports to NCI, an equilibrium solubility in water as 0.6 mg/mL was noted. Recently, two batches of bortezomib were purchased from a commercial source (see materials), analyzed and solubility reassessed in light of the confusion seen in the literature. Purity analysis based on HPLC of the two batches showed both to be pure and free of known major degradation products and impurities.

The initial solubility attempts in water with the first batch gave consistent values of about 0.6 mg/mL after both 24 hr and 48 hr in a 25°C in a temperature controlled orbital shaker incubator. The second batch provided an initial apparent solubility of about 3.5 mg/mL after 24 hours dropping to about 0.6 mg/mL at 48 hours. This is consistent with the material being amorphous.

All subsequent solubility determinations gave the lower, consistent solubility after 24 hours incubation.

When subjected to DSC and TGA analysis, both solid sample batches appear to have been the monomer and not the boroxine as an endothermic melting peak at 180°C was seen only after a weight loss of about 4.5%, beginning at about 50°C, corresponding to three water molecules, Fig. 2. This endotherm was not seen every time a DSC was run. When observed, this is consistent with boroxine formation and crystallization in some samples as illustrated in Fig. 1. That is, the melt at 180°C is due to that of the boroxine formed on dehydration of monomeric bortezomib. This behavior is similar to that reported from this laboratory on the model aryl boronic, 4-methoxybenzeneboronic acid (4-MBBA).[10]

Insert Figure 2

The logarithmic solubility of bortezomib as a function of solution pH, measured at the end of the experiment, and not initial pH, is shown in Fig. 3. As with 4-MBBA, this curve is consistent with the solubility behavior of a weak acid, in this case, a weak Lewis acid.[10] The solid line is the fit of the data to Eq. 1 for the pH dependency of weak acid where the solubility is pH independent below the pKa of the acid and then increases above the pKa as the corresponding boronate is formed. By fitting the experimental data to Eq. 1,[10] the intrinsic solubility (S_0) of the bortezomib is about 0.54 ± 0.4 mg/mL and the pKa of bortezomib under these conditions is 8.8 ± 0.2,

$$S = S_0 + \frac{K_a S_0}{[H^+]} \qquad (1)$$

where S is the measured solubility, S_0 is the equilibrium solubility of the neutral form of the acid, Ka is the dissociation constant of the acid and $[H^+]$ is the measured hydrogen ion concentration from the pH measurement.

Insert Figure 3

The second, higher curve shown in Fig. 3 is that for bortezomib in the presence of 500 mM D-mannitol. Unlike 4-MBBA, in this case, apparent formation of the D-mannitol ester/s for bortezomib as described by Gupta et al., results in a significant increase in solubility.[2,6,7] A fit of this data to Eq. 1 gives an apparent equilibrium solubility of 5.2 ± 0.4 mg/mL and an apparent pKa of 7.2 ± 0.2. The drop in pKa, although smaller than noted in earlier papers from our studies for other boronic acids, is consistent with prior literature for boronic acids in the presence of 1,2-diols and polyols.[11-14]

Fig. 4 shows the solubility of bortezomib at 25°C as a function of D-mannitol concentration at pH 5.5-6.5, below the pKa value for bortezomib and its D-mannitol ester. Noted on this plot is the solubility of bortezomib in the presence of the same D-mannitol concentration (55 mM) when a Velcade® formulation is diluted for IV administration to 1 mg/mL bortezomib. The actual bortezomib measured solubility is about 1.8 mg/mL, well in excess of 1 mg/mL. If diluted to 2.5 mg/mL for SC administration (137 mM D-mannitol concentration), the solubility is about 3.2 mg/mL, again in excess of 2.5 mg/mL needed. Moreover, similar results are observed in NS as well (Table 1).

Insert Fig. 4

Insert Table 1

A number of studies have been reported that reconstituted samples of Velcade® stored at refrigerator temperatures for times longer than recommended by the manufacturer, remain clear and free of precipitate/particulates suggesting that the higher than needed solubility has the added benefit of allowing storage at temperatures lower than 25°C at least for >24 hours without precipitate formation.[15-18]

An alternative formulation of bortezomib to the Velcade® formulation by Fresenius Kabi LLC (USA), utilizes boric acid and glycine in its freeze-dried product.[8] This formulation can be reconstituted to 1 mg/mL bortezomib for further dilution for IV administration. The equilibrium solubility of bortezomib in the presence of 3 mg/mL boric acid, 10 mg/mL glycine and 3 mg/mL boric acid and 10 mg/mL glycine, corresponding to the concentrations in the Fresenius Kabi USA, LLC formulation are 0.58 ± 0.06, 0.57 ± 0.09, 0.58 ± 0.07 mg/mL, respectively. That is, once diluted, the presence of boric acid and glycine does not enhance the equilibrium solubility of bortezomib in NS. In the patent covering the freeze-dried formulation, the inventors claim that their formulation prevents formation of the crystalline bortezomib and its poorly soluble boroxine resulting in rapid reconstitution times.[19] However, from our solubility measurements, the reconstituted product provides a metastable formulation as 1 mg/mL is in excess of the intrinsic solubility of bortezomib under the formulation conditions. In defense of the formulation, since bortezomib is said to be in it as an amorphous and not as its boroxine form, reconstitution of the freeze-dried cake to 1 mg/mL is very reasonable.

CONCLUSION

This paper provides clarity on the solubility characteristics of the novel boronic acid drug, bortezomib. Bortezomib equilibrium solubility in water and NS is 0.5-0.6 mg/mL but higher initial, kinetic solubility is occasionally seen when a non-crystalline bortezomib source is used for study. However, on exposure at 25°C, the solubility drops to an equilibrium solubility of 0.5-0.6 mg/mL. D-mannitol increases the solubility of bortezomib in a non-linear fashion with increasing concentrations due to reversible ester formation providing an equilibrium solubility in NS of 1.92 ± 0.14 and 3.40 ± 0.21 mg/mL at respectively 55 and 137 mM D-mannitol concentrations used to

reconstitute the commercial Velcade® product. The solubility-pH profile of bortezomib allows one to estimate a pKa value for bortezomib of 8.8 ± 0.2. In the presence of 500 mM D-mannitol, the apparent pKa of bortezomib is estimated to be 7.2 ± 0.2. Boric acid, glycine and a combination at concentrations used in the alternative formulation to Velcade® do not increase the equilibrium solubility of bortezomib.

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REFERENCES

1. Adams J and Kauffman M. *Cancer Invest.* Development of the proteasome inhibitor Velcade™ (Bortezomib). 2004; 22(2):304-311.

2. Plamondon L, Grenier L, Adams J, Gupta SL. Formulation of boronic acid compounds. 2004. Patent US 6699835.

3. Velcade, Highlights of Prescribing Information, https://www.velcade.com/Files/PDFs/VELCADE_PRESCRIBING_INFORMATION.pdf.

4. Tan CRC, Abdul-Majeed S, Cael B, Barta SK. Clinical pharmacokinetics and pharmacodynamics of bortezomib. *Clin Pharmacokinet*. 2019; 58(2):157-168.

5. Deshantri AK, Metselaar JM, Zagkou S, Storm G, Mandhane SN, Fens MH, Schiffelers RM. Development and characterization of liposomal formulation of bortezomib. *Int J Pharm: X*. 2019; 1:100011.

6. Gupta SL, Stella VJ, Waugh W. Formulation of boronic acid compounds. 2004. Patent US 6713446.

7. Gupta SL, Stella VJ, Waugh W. Formulation of boronic acid compounds. 2005. Patent US 6958319.

8. Fresenius Kabi. Bortezomib for injection. 2018. Available at: http://editor.fresenius-kabi.us/PIs/US-PH-Bortezomib_FK-451289C_Sep_2018-PI.pdf.

9. Wu S, Waugh W, Stella VJ. Degradation pathways of a peptide boronic acid derivative, 2-Pyz-(CO)-Phe-Leu-B $(OH)_2$. *J Pharm Sci*. 2000; 89(6):758-765.

10. Lopalco A, Marinaro WA, Day VW, Stella VJ. Isolation, solubility, and characterization of D-mannitol esters of 4-methoxybenzeneboronic acid. *J Pharm Sci*. 2017; 106(2):601-610.

11. Marinaro WA, Schieber LJ, Munson EJ, Day VW, Stella VJ. Properties of a model aryl boronic acid and its boroxine. *J Pharm Sci*. 2012; 101(9):3190-3198.

12. Marinaro WA, Prankerd R, Kinnari K, Stella VJ. Interaction of model aryl-and alkyl-boronic acids and 1, 2-diols in aqueous solution. *J Pharm Sci*. 2015; 104(4):1399-1408.

13. Lopalco A, Stella VJ, Thompson WH. Origins, and formulation implications, of the pKa difference between boronic acids and their esters: A density functional theory study. *Eur J Pharm Sci*. 2018; 124:10-16.

14. Lopalco A, Lopedota AA, Laquintana V, Denora N, Stella VJ. Boric acid, a Lewis acid with unique and unusual properties: formulation implications. *J Pharm Sci*. 2020; 109(8):2375-2386.

15. André P, Cisternino S, Chiadmi F, Toledano A, Schlatter J, Fain O, Fontan JE. Stability of bortezomib 1-mg/mL solution in plastic syringe and glass vial. *Ann Pharmacother*. 2005; 39(9):1462-1466.

16. Sánchez-Serrano I. Success in translational research: lessons from the development of bortezomib. *Nat Rev Drug Discov*. 2006; 5(2):107-114.

17. Vanderloo JP, Pomplun ML, Vermeulen LC, Kolesar JM. Stability of unused reconstituted bortezomib in original manufacturer vials. *J Onc Pharm Pract*. 2011; 17(4):400-402.

18. Walker SE, Charbonneau LF, Law S. Stability of bortezomib 2.5 mg/mL in vials and syringes stored at 4°C and room temperature (23 C). *Can J Hosp Pharm*. 2014; 67(2):102.

19. Usayapant A, Bowman D. Bortezomib formulations. 2015. Patent US 8962572.

Table 1. Solubility of bortezomib (mg/mL) and standard deviation (S.D.) in water and 0.9% NaCl without and with 55 mM (10 mg/mL) and 137 mM (25 mg/mL) D-mannitol.

	Bortezomib solubility (mg/mL) ± S.D.	
D-Mannitol concentration (mM)	Water	0.9% NaCl
0	0.59 ± 0.07	0.52 ± 0.11
55	1.84 ± 0.10	1.92 ± 0.14
137	3.18 ± 0.17	3.40 ± 0.21

Figures

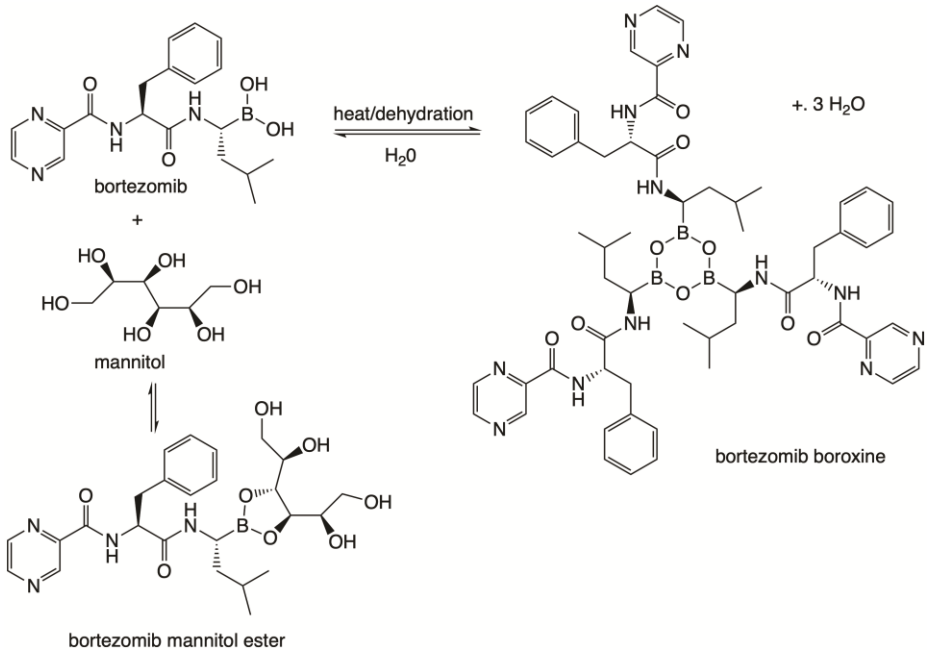

Figure 1. The structures of bortezomib, its boroxine, D-mannitol and the presumed primary structure of the D-mannitol ester of bortezomib. The figure also shows how on dehydration, bortezomib forms the corresponding boroxine, or bortezomib anhydride, with the loss of three water molecules.[4,18]

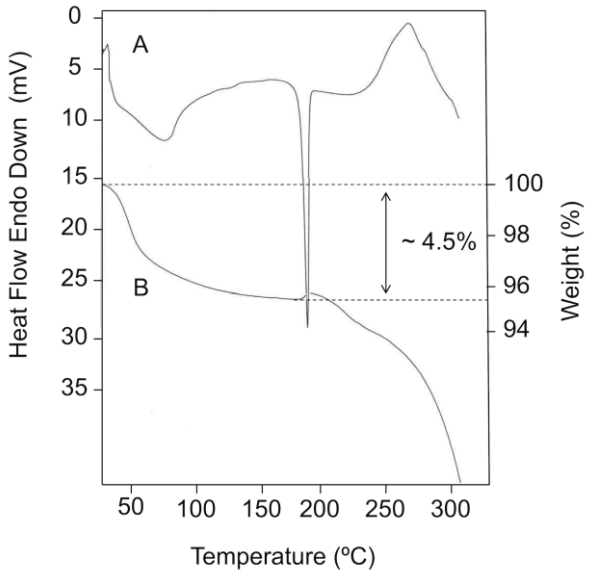

Figure 2. DSC (A) and TGA (B) plots for bortezomib showing the loss of three moles of water (~4.5%) beginning at about 50°C and a large endothermic peak at 180°C corresponding to the probable melt of the formed bortezomib boroxine formed during the dehydration.

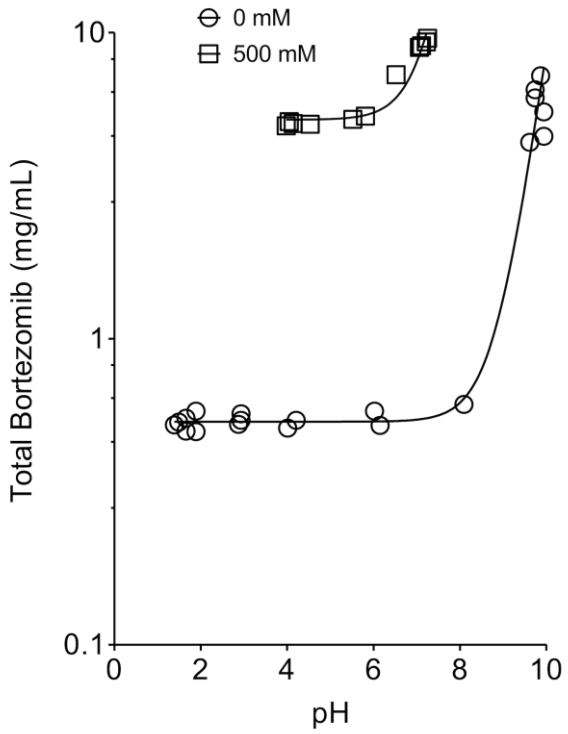

Figure 3. pH-Solubility profile for bortezomib in water (circles) and in the presence of 500 mM D-mannitol (squares) at 25°C.

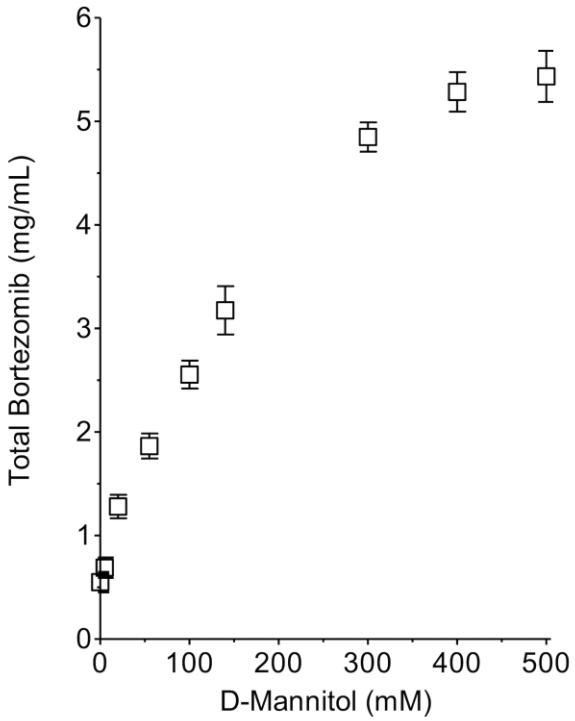

Figure 4. Solubility of bortezomib in the presence of increasing D-mannitol concentration in water at pH 5.5-6.5.